Amendments to the Specification:

At page 1, please delete the existing title and enter the new title of the application as follows:

"NUCLEIC ACIDS FOR DETECTING COLON CANCER RELATED GENE (CCRG) AND METHODS OF USE"

At page 1, please replace the first paragraph with the following amended paragraph:

The present application is a divisional application of U.S. Serial No. 09/730,212 filed December 5, 2000, and issued January 13, 2004 as U.S. Patent No. 6,677,119, which claims the benefit of U.S. Provisional Application No. 60/200, 292, filed April 28, 2000.

At page 2, please replace the paragraph at the bottom of the page with the following amended paragraph:

An open reading frame of the CCRG gene encodes a polypeptide, i.e., the CCRG protein, which was predicted to have a signal peptide sequence, and putative phosphorylation, myristylation, and glycosylation sites. Based on comparisons to sequences of known function, the nucleotide sequence of CCRG (and C4) was predicted to encode a prokaryotic lipoprotein binding site and a prenylation site. The C-terminus of the CCRG protein is cysteine rich and contains a motif found in ultra high sulphur matrix protein, hair keratin, metallothionein and cation transporters. Using the secondary structure prediction program provided by the ExPASy proteomics server

~~(http://www.expasy.ch)~~ by the Swiss Institute of Bioinformatics (Geneva)[[.]]. CCRG protein was predicted to contain mostly a mixture of alpha helices, beta strands, and coils. The mature CCRG protein has a theoretical molecular weight of 8.62 kDa and a pI of 8.05. These and other analyses indicated that CCRG protein is a colon tumor associated secreted factor.

At page 8 line 7, please replace the third paragraph with the following amended paragraph:

As used herein, a "nucleic acid" or a "nucleic acid molecule" means a chain of two or more nucleotides such as RNA (ribonucleic acid) and DNA (deoxyribonucleic acid). A "purified" nucleic acid molecule is one that has been substantially separated or isolated away from other nucleic acid sequences in a cell or organism in which the nucleic acid naturally occurs (e.g., 30, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 100% free of contaminants). The term includes, e.g., a recombinant nucleic acid molecule incorporated into a vector, a plasmid, a virus, or a genome of a prokaryote or eukaryote. Examples of purified nucleic acids include cDNAs, fragments of genomic nucleic acids, nucleic acids produced by polymerase chain reaction (PCR), nucleic acids formed by restriction enzyme treatment of genomic nucleic acids, recombinant nucleic acids, and chemically synthesized nucleic acid molecules. A "recombinant" nucleic acid molecule is one made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

At page 9, line 4, please replace the second paragraph with the following amended paragraph:

As used herein, "protein" or "polypeptide" are used synonymously to mean any peptide-linked chain of amino acids, regardless of length or post-translational modification, e.g., glycosylation or phosphorylation. ~~An~~ A "purified" polypeptide is one that has been substantially separated or isolated away from other polypeptides in a cell or organism in which the polypeptide naturally occurs (e.g., 30, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 100% free of contaminants). By the terms "CCRG gene," "CCRG polynucleotide," or "CCRG nucleic acid" is meant a native CCRG-encoding nucleic acid sequence, e.g., the native CCRG cDNA (as shown in FIG. 6); a nucleic acid having sequences from which CCRG cDNA can be transcribed; and/or allelic variants and homologs of the foregoing. The terms encompass double-stranded DNA, single-stranded DNA, and RNA.

At page 9, line 10, please replace the third paragraph with the following amended paragraph:

By the terms "CCRG protein" or "CCRG polypeptide" is meant an expression product of ~~an~~ a CCRG gene such as the native CCRG protein of Fig. 7 (SEQ ID NO:7) or FIG. 8 (amino acid residues 31-11 of SEQ ID NO:7) or a protein that shares at least 65% (but preferably 75, 80, 85, 90 , 95, 96, 97 ,98, or 99%) amino acid sequence identity with the protein of FIG. 7 or FIG. 8 and displays a functional activity of CCRG. A "functional activity" of a protein is any activity associated with the physiological function of the protein. For example, functional activities of CCRG may include selective

expression in certain neoplastic tissues. In addition, the expression of CCRG in the small intestine suggests that it may be an autocrine secreted growth factor in the intestine and that its overexpression in the large intestine (colon) may contribute to tumor formation.

At page 10, line 15, replace the third paragraph with the following amended paragraph:

When referring to hybridization of one nucleic to another, "low stringency conditions" means in 10% formamide, 5X ~~Denhart's~~ Denhardt's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C; "moderate stringency conditions" means in 50% formamide, 5X ~~Denhart's~~ Denhardt's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C; and "high stringency conditions" means in 50% formamide, 5X ~~Denhart's~~ Denhardt's solution, 5X SSPE, 0.2% SDS at 42°C., followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C. The phrase "stringent hybridization conditions" means low, moderate, or high stringency conditions.

At page 11, line 2, please replace the first paragraph with the following amended paragraph:

As used herein, "sequence identity" means the percentage of identical subunits at corresponding positions in two sequences when the two sequences are aligned to maximize subunit matching, i.e., taking into account gaps and insertions. When a subunit position in both of the two sequences is occupied by the same monomeric subunit, e.g., if a given position is occupied by an adenine in each of two DNA molecules, then the

molecules are identical at that position. For example, if 7 positions in a sequence of 10 nucleotides in length are identical to the corresponding positions in a second l0-nucleotide sequence, then the two sequences have 70% sequence identity. As another example, if 12 positions in a protein sequence 20 amino acids in length are identical to the corresponding positions in a second 20-amino acid sequence, then the two sequences have 60% sequence identity. Preferably, the length of the compared nucleic acid sequences is at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides[[;]], and the length of compared polypeptide sequences is at least 15, 25, and 50 amino acids. Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

At page 14, line 18, begin a new paragraph (i.e., divide the text from the paragraph it is in, starting with the sentence beginning "Although methods and materials...," as indicated in the following two amended paragraphs:

The term "labeled," with regard to a probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the particular embodiments discussed below are illustrative only and not intended to be limiting.

At page 15, line 12, please replace the fourth paragraph with the following amended paragraph:

FIG. 7 is the amino acid sequence of the ~~processed~~ unprocessed form (i.e., ~~without~~ with the signal peptide) of native CCRG protein.

At page 15, line 14, please replace the first paragraph with the following amended paragraph:

FIG. 8 is the amino acid sequence of the ~~unprocessed~~ processed form (i.e., ~~with~~ without the signal peptide) of native CCRG protein.

At page 17, line 10, please replace the second paragraph with the following amended paragraph:

The present invention utilizes the human CCRG gene, which has now been cloned and sequenced. A preferred nucleic acid molecule for use in the invention is the native CCRG polynucleotide shown in FIG. 6 (SEQ ID NO:6) and deposited with Genbank as

Accession No. AF323921. The clone G6 containing the full length CCRG gene (SEQ ID NO:6) in the PEAK 8 expression vector (Edge Biosystems) ~~has been~~ was deposited with the American Type Culture Collection (Rockville, MD) as Accession No. PTA-2739 on November 30, 2000. Another nucleic acid that can be used in various aspects of the invention includes a purified nucleic acid (polynucleotide) that encodes a polypeptide having either the amino acid sequence of Fig. 7 (SEQ ID NO:7) or the amino acid sequence of Fig. 8 (amino acid residues 31-111 of SEQ ID NO:7). As the native CCRG gene was originally cloned from a small intestine, cDNA library nucleic acid molecules encoding a polypeptide of the present invention can be obtained from such a library or from any human colon tumor tissue itself by conventional cloning methods such as those described herein.

At page 20 line 1, please replace the first paragraph with the following amended paragraph:

Non-naturally occurring CCRG gene variants are nucleic acids that do not occur in nature (e.g., are made by the hand of man), have at least 75% (e.g., 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and 99%) sequence identity with the native CCRG gene, and encode polypeptides having structural similarity to native CCRG protein. Examples of non-naturally occurring CCRG gene variants are those that encode a fragment of a CCRG protein, those that hybridize to the native CCRG gene or a complement of [[to]] the native CCRG gene under stringent conditions, those that share at least 65% sequence

identity with the native CCRG gene or a complement of the native CCRG gene, and those that encode a CCRG fusion protein.

At page 22 line 8, please replace the second paragraph with the following amended paragraph:

Using the nucleotide of the native CCRG gene and the amino acid sequence of a native CCRG protein, those skilled in the art can create nucleic acid molecules that have minor variations in their nucleotide sequences, by, for example, standard nucleic acid mutagenesis techniques or by chemical synthesis. Variant CCRG nucleic acid molecules can be expressed to produce variant CCRG proteins.

At page 27 line 5, please replace the second paragraph with the following amended paragraph:

Because it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation [[on]] of endogenous mRNAs, a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong promoter (e.g., the CMV promoter). The use of such a construct to transform cells will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous CCRG transcripts and thereby prevent translation of CCRG mRNA.

At page 30 line 12, please replace the third paragraph with the following amended paragraph:

The invention also includes oligonucleotide probes (i.e., isolated nucleic acid molecules conjugated with a detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, or enzyme)[[;]], and oligonucleotide primers (i.e., isolated nucleic acid molecules that can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase). Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other conventional [[nucleic-acid]] nucleic acid amplification methods. Probes and primers within the invention are generally 15 nucleotides or more in length, preferably 20 nucleotides or more, more preferably 25 nucleotides, and most preferably 30 nucleotides or more. Preferred probes and primers are those that hybridize to the native CCRG gene sequence under high stringency conditions, and those that hybridize to CCRG gene homologs under at least moderate stringency conditions. Preferably, probes and primers according to the present invention have complete sequence identity with the native CCRG gene sequence, although probes differing from the native CCRG gene sequence and that retain the ability to hybridize to native CCRG gene sequences under stringent conditions may be designed by conventional methods. Primers and probes based on the native CCRG gene sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed native CCRG gene sequence by conventional methods, e.g., by re-cloning and sequencing a native CCRG cDNA. Particularly preferred primer pairs for use in the invention are shown as SEQ ID NO:2 and SEQ ID NO:3; and SEQ ID NO:9 and SEQ ID NO:10, both pairs having been shown to selectively amplify CCRG gene sequences, the former

amplifying a 455 bp product, the latter amplifying a 267 bp product including the signal sequence and most of the ~~CDS~~ coding sequence of the CCRG gene. A particularly preferred oligonucleotide probe for use in the invention is shown as SEQ ID NO:4.

At page 43 line 8, please replace the second paragraph with the following amended paragraph:

Additionally, methods can be employed that result directly in the identification of genes that encode proteins that interact with a CCRG protein. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of [[l]] λgt11 libraries, using a labeled CCRG protein or a CCRG fusion protein, for example, a CCRG protein or domain fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

At page 48, line 2, please replace the first paragraph with the following amended paragraph:

Unigene [[Hs.105470]] Hs 105470 was identified as being present in the colon tumor tissues, but not in the normal tissue. Total RNA was isolated from a matched set of normal and colon tumors and reverse transcribed using random hexamers and Superscript reverse transcriptase (Life Technologies, Rockville, MD). One-fortieth of the resulting cDNAs was PCR-amplified using the PCR primers described herein as SEQ ID Nos: 2 and 3. The conditions for the PCR included 1) initial denaturation at 94°C for 7 mins; 2) denaturation at 94°C for 1 min, annealing at 62°C for 2 mins[[. And]] and extension at 72°C for 3 mins, for 35 cycles with a final extension at 72°C for 10 mins.

Referring to FIG. 1, Unigene Hs 105470 showed a RT-dependant PCR product of 455 bp. This product was not seen in the control RT-minus reaction, nor in peripheral blood lymphocyte DNA. A product of higher molecular weight was detected in the genomic DNA sample, indicating that the RT-PCR primers reside in two different exons. UniGene # 105470 has five ESTs assigned to the cluster. The sequence of the longest EST (Genbank Accession No. AA524300) in this UniGene is 577 bp in length (which was the maximum size extendable as a contig) and is shown herein as SEQ ID NO:1. The RT-PCR primers used to identify a gene encompassing this EST, termed CCRG, ~~is~~ are shown herein as SEQ ID NO:2 (sense) and SEQ ID NO:3 (antisense).

At page 50, please replace the second paragraph with the following amended paragraph:

Four independent clones strongly hybridizing to the probe were selected for sequencing. Sequencing was done using pEAK8 forward (5' GGA TCT TTG GTT CAT TCT CAA 3' SEQ ID NO: 11) and pEAK8 reverse (5' CTG GAT GCA GGC TAC TCT AG 3' SEQ ID NO:12). Both of these primers are present outside the cloning sites in the poly linker region of the pEAK8 vector. All the four clones contained additional sequences from the nucleic acid of SEQ ID NO:5. One of the ~~clone~~ clones, termed G6, contained a complete open reading frame with a signal peptide sequence. The ~~g6~~ G6 clone had an insert size of approximately 800 bp and detected a mRNA of about 750 bp in a Northern blot of colon tumor-derived RNA, but not in the normal colon mRNA. RT-PCR primers encompassing the entire G6 clone also detected a specific product in the colon tumor derived mRNAs, but not in the corresponding normal colon derived mRNAs.

The G6 clone also contained a polyadenylation site and a poly A tail. The gene thus identified was termed CCRG for Colon Carcinoma Related Gene.

At page 51, please replace the first paragraph in Example 3 with the following amended paragraph:

The CCRG gene has a signal peptide sequence M G P S S C L L L I L I P L L Q L I N P G S T Q C S L D S V (SEQ ID NO:13) upstream of the initiation Met codon. This consensus signal peptide sequence is found in secreted growth factors and cytokines. Using the SignaP prediction program at the Swiss Expasy site (~~http://www.expasy.ch/~~), the precise position for the signal peptidase cleavage of the CCRG gene is predicted to occur at GST-QC leaving a leader sequence of 7 amino acids before the Met codon of the mature CCRG protein. The PSORT program at the Expasy site which predicts the cell localization predicted that the CCRG gene is likely localized outside the cell. The mature protein has a theoretical MW of 8.62 kDa and pI of 8.05.

At page 51, please replace the paragraph at the bottom of the page with the following amended paragraph:

Nucleotide and amino acid homology searches at the NCBI (~~http://www.ncbi.nlm.nih.gov/BLAST/~~) revealed no significant homology to known proteins. Analysis of motifs and patterns at the ProCyte database (~~http://www.expasy.ch/tools/scnpsite.html~~) of the Expasy site showed that the CCRG gene product likely encodes phosphorylation sites, myristylation sites, and glycosylation

sites. In addition, a prokaryotic lipoprotein binding site and a prenylation site were identified as being encoded by the CCRG gene.

At page 53, line 16, please replace the third paragraph with the following amended paragraph:

The CCRG gene was detected using ~~of~~ an oligonucleotide probe labeled with ^{32}P-labeled dNTP. An oligonucleotide corresponding to SEQ ID NO:4 was synthesized, and then end-labeled with gamma ^{32}P-labeled dATP using polynucleotide kinase. RT-PCR products were generated in the presence or absence of RT from a matched set of tumor and normal colon, transferred to a nitrocellulose membrane, and hybridized to the ^{32}P-labeled oligonucleotide probe. As shown in FIG. 5, this probe hybridized to a 455 bp product in the [[tumor derived]] tumor-derived cDNA, but not in the normal tissue cDNA. The probe also detected a band in a genomic DNA (ca.1.5 kbp) sample obtained from peripheral blood lymphocytes.

At page 54, line 15, please replace the third paragraph with the following amended paragraph:

In addition, a method for diagnosing colon cancer using blood or blood-derived materials (e.g., serum) using ~~the~~ antibodies to the CCRG gene is ~~an~~ envisioned as the CCRG protein is predicted to be a secreted protein. CCRG protein levels above the baseline due to the production of the CCRG protein by intestine cells would be indicative of colon cancer in the ~~patients~~ patient. The levels of secreted CCRG protein in the

serum/plasma can be measured by methods described elsewhere herein including, e.g., by Enzyme Linked Immunosorbent assay (ELISA) or Western blotting.

At page 55, line 2, please replace the first paragraph with the following amended paragraph:

Using hybridization techniques, CCRG gene expression can be detected with the [[oligo-nucleotide]] oligonucleotide probe described herein as SEQ ID NO:4. The oligonucleotide is labeled with radioactive or non-radioactive nucleotides, and the labeled probe is reacted with RNA from the sample being analyzed in the form of a Northern blot by transferring the products onto a filter (for example, nitrocellulose). This method can also be performed in the form of Southern blot of RT-PCR reaction products made from the genomic DNA contained in a sample being analyzed. Following hybridization to the oligonucleotide probe, the filter is washed, exposed to X-ray film, and [[auto-radiographed]] autoradiographed. Bands that hybridize to the probe can be identified from the autoradiogram. The oligonucleotide probe can also be used for in situ hybridization reactions to directly detect CCRG gene expression in tissues.

At page 56, line 10, please replace the second paragraph with the following amended paragraph:

Inhibition of CCRG gene expression can be accomplished using an antisense nucleic acid. For example, as described above, a suitable length (e.g., 18-25 bases) of an antisense nucleic acid that specifically hybridizes to the 5' or 3' non-coding region of the CCRG gene is synthesized, and then introduced into target tissues or cells (e.g., by

electroporation or delivery via a vector) or liposomes. The target tissues or cells are then placed under conditions that allow the [[anti-sense]] antisense nucleic acid to hybridize to the mRNAs transcribed from the CCRG gene. This hybridization prevents translation and thereby [[to]] selectively inhibits expression of CCRG protein. See, e.g., Narayanan, R. In Vivo, 8: 787-794, 1994. As another example, the foregoing antisense nucleic acid can also be generated as a stable recombinant construct that can be delivered in vivo for gene therapy. See, e.g., Higgins et al., Proc Nat'l Acad Sci USA 90: 9901-9905, 1993.

At page 58, line 3, please replace the second paragraph with the following amended paragraph:

As CCRG is selectively expressed in colon tumors [[;]], but not in a variety of other tumors, compounds can be screened for the ability to selectively inhibit growth of CCRG-expressing tumors. Compounds identified in this manner can be further evaluated for CCRG-specific inhibition using the CCRG promoter-reporter gene constructs described above.